National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 3667

Creatinine in Frozen Human Urine

This Standard Reference Material (SRM) is intended primarily for use in evaluating the accuracy of procedures for the determination of creatinine in human urine. It is also intended for use in validating working or secondary reference materials. SRM 3667 was prepared from normal human urine collected from male and female donors, and the creatinine concentration has not been modified. A unit of SRM 3667 consists of one bottle of 10 mL frozen human urine.

Certified Values: The certified mass fraction value and mass concentration value for creatinine are provided in Table 1. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1]. The certified values for creatinine are based on the results from a modification of the NIST isotope dilution liquid chromatography mass spectrometry (ID-LC-MS) method for the determination of creatinine in serum [2]. This method is recognized as a higher-order reference measurement procedure by the Joint Committee for Traceability in Laboratory Medicine (JCTLM) [3].

Expiration of Certification: The certification of **SRM 3667** is valid, within the measurement uncertainty specified, until **31 January 2018**, provided the SRM is handled and stored in accordance with instructions given in this certificate (see "Instructions for Storage and Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Coordination of the technical measurements leading to the certification of SRM 3667 was performed by K.W. Phinney of the NIST Biomolecular Measurement Division and J.E. Camara of the NIST Chemical Sciences Division.

Acquisition of the material was performed by K.W. Phinney. Certification measurements were performed by J.E. Camara. Additional measurements in support of the development of SRM 3667 were performed by L.T. Sniegoski of the NIST Chemical Sciences Division.

Statistical consultation was provided by N.F. Zhang of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

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Certificate Revision History on Last Page

Steven J. Choquette, Acting Director
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NOTICE AND WARNINGS TO USERS

SRM 3667 IS INTENDED FOR RESEARCH USE. THIS IS A HUMAN-SOURCE MATERIAL. HANDLE PRODUCT AS A BIOHAZARDOUS MATERIAL CAPABLE OF TRANSMITTING INFECTIOUS DISEASE. The supplier of this urine has reported that the pooled urine was tested and found to be non-reactive/negative for *Escherichia coli*, *Salmonella*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. However, no known test method can offer complete assurance that infectious agents are absent from this material. Accordingly, this human urine-based product should be handled at the Biosafety Level 2 or higher as recommended for any POTENTIALLY INFECTIOUS HUMAN SPECIMEN in the Centers for Disease Control and Prevention/National Institutes of Health Manual [4].

INSTRUCTIONS FOR STORAGE AND USE

Storage: The SRM is stored at –80 °C at NIST. The urine is shipped frozen (on dry ice) and, upon receipt, should be stored frozen until ready for use. A freezer temperature of –20 °C is acceptable for storage for up to one week. If a longer storage time is anticipated, the material should be stored at or below –60 °C. The SRM should not be exposed to sunlight or ultraviolet radiation. Storage of thawed material at room or refrigerator temperatures may result in changes in analyte concentrations.

Use: Vials of the SRM to be analyzed should be removed from the freezer and thawed to room temperature (20 °C to 25 °C). After the material is thawed to room temperature, it should be used immediately. The material should be swirled gently to mix it before aliquots are withdrawn.

SOURCE, PREPARATION, AND ANALYSIS[(1)]

Source and Preparation: SRM 3667 was prepared by Solomon Park Research Laboratories (Kirkland, WA). The urine pool was prepared from a minimum of 10 donors, with both male and female donors included.

Analysis: Value assignment of the concentration of creatinine in SRM 3667 was based on the results from a modification of the NIST ID-LC-MS reference measurement procedure for creatinine in serum [2]. SRM 914a *Creatinine* was used to calibrate the method and creatinine-d_3 was used as the internal standard. Aliquots of urine were combined with internal standard solution and diluted with water and 1 mol/L HCl to achieve approximately 1:10 dilution (volume fractions) of the urine sample and an HCl concentration of 0.01 mol/L. Solutions were vortexed and allowed to equilibrate overnight. A portion of the sample solution was combined with 0.01 mol/L HCl to achieve a dilution of 1:100 (volume fractions) compared to the original urine sample. Samples were analyzed by LC-MS with electrospray ionization in the positive ion mode. Selected ion monitoring (SIM) was used to detect creatinine at *m/z* 114 and creatinine-d_3 at *m/z* 117.

Homogeneity Analysis: The homogeneity assessment was made at the time the certification analyses were performed. A stratified sampling plan was devised to test for homogeneity across the lot of bottles. There was no apparent trend in the data when plotted against the sequence in which the bottles were prepared.

Certified Values: The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence; it incorporates Type B uncertainty components related to the analyses, consistent with the ISO/JCGM Guide and with its Supplement 1 [5,6]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is the combined uncertainty, and k is a coverage factor corresponding to approximately 95 % confidence for each analyte [5]. For the certified values shown below, $k = 2$. The measurand is the total mass fraction of creatinine as listed in Table 1. Metrological traceability is to the SI derived unit for mass fraction (expressed as micrograms per gram) and mass concentration (milligrams per deciliter).

Table 1. Certified Values for Creatinine in SRM 3667

Mass Fraction (µg/g)	Mass Concentration[(a)] (mg/dL)
613 ± 13	61.8 ± 1.3

[(a)] Mass concentration was calculated from the mass fraction using the measured urine density, 1.00816 g/mL. The uncertainty in the urine density measurements was incorporated in the value that is reported relative to units of volume.

[(1)] Certain commercial equipment, instrumentation, or materials are identified in this certificate to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

REFERENCES

[1] May, W.; Parris, R.; Beck, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260–136; U.S. Government Printing Office: Washington, D.C. (2000); available at http://www.nist.gov/srm/publications.cfm (accessed Jan 2016).

[2] Dodder, N.G.; Tai, S.S.C.; Sniegoski, L.T., Zhang, N.-F.; Welch, M.J.; *Certification of Creatinine in a Human Serum Reference Material by GC-MS and LC-MS*; Clin. Chem., Vol. 53, pp. 1694–1699 (2007).

[3] Joint Committee for Traceability in Laboratory Medicine; available at http://www.bipm.org/en/committees/jc/jctlm/ (accessed Jan 2016).

[4] CDC/NIH; *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed.; Richardson, J.; Barkley, W.E.; Richmond, J.; McKinney, R.W., Eds.; U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health; US Government Printing Office: Washington, D.C. (2009); available at http://www.cdc.gov/biosafety/publications/index.htm (accessed Jan 2016).

[5] JCGM 100:2008; *Evaluation of Measurement Data - Guide to the Expression of Uncertainty in Measurement* (GUM 1995 with Minor Corrections); Joint Committee for Guides in Metrology (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Jan 2016); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297; U.S. Government Printing Office: Washington, DC (1994); available at http://physics.nist.gov/Pubs/ (accessed Jan 2016).

[6] JCGM 101:2008; *Evaluation of Measurement Data – Supplement 1 to the Guide to the Expression of Uncertainty in Measurement – Propagation of Distributions Using a Monte Carlo Method*; JCGM (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_101_2008_E.pdf (accessed Jan 2016).

Certificate Revision History: 21 January 2016 (Editorial changes); 20 March 2013 (Original certificate date).

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.